Posiphen: Experimental Alzheimer Agent that Lowers Amyloid Precursor Protein Levels in Culture and In Vivo

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Abstract

Major hallmarks of Alzheimer's disease (AD) are synaptic loss and abnormal protein deposition, particularly amyloid plaque and neurofibrillary tangles. Current AD drugs on the market today provide symptomatic relief and improve cognition - cholinesterases inhibitors (ChE-Is) and memantine are the only FDA approved drugs for AD.

Posiphen® tartrate, an inhibitor of amyloid precursor protein (APP) synthesis[1], is being developed by QR Pharma as a potential disease modifying treatment for Alzheimer's disease. Through APP inhibition, Posiphen may halt or slow disease progression by reducing β amyloid (Aβ), the substrate available for formation of toxic oligomers. Evidence in the literature suggests that targeting the accumulation of Aβ, a hydrophobic, neurotoxic self-aggregating 40 to 42 amino acid peptide that accumulates preferentially within amyloid plaques in the brain, could change the course of AD[2]. Other data from Genetech show that APP in the absence of trophic factors is shed from the surface of neuronal cells and processed into an amino terminal fragment (N-APP) that binds to DR6 receptors and induces nerve cell death[3]. The Bredesen Lab identified another factor that is cleaved from the C-terminal end of APP (C31) and causes nerve cell degeneration and death in tissue culture cells and in transgenic mice[4].

In all three cases , reducing APP synthesis could be beneficial to the brain, because through the Aβ pathway neurotoxic plaque is reduced and through the inhibition of N- and C- terminal fragments nerve cell death is inhibited and brain cells are preserved. Ongoing studies are elucidating the mode of action of Posiphen and its analogs on the APP processing pathway. Posiphen has proven well tolerated in initial phase 1 clinical trials and achieves concentrations associated with substantial inhibition of APP and Aβ in animal models. These results suggest that Posiphen and analogs are promising experimental drugs for diseases where lowered brain APP levels contribute to the progression of the disease.

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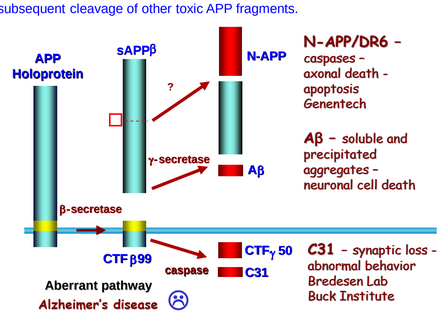
Introduction

- Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that results in debilitating dementia. Whereas anticholinesterases provide symptomatic relief, there are no approved drugs that impact disease progression.
- AD pathology is characterized by amyloid-β peptide (Aβ) protein deposition and neurofibrillary tangles, combined with massive synaptic and neuronal loss [5,6]. These are the primary targets of current AD drug design[7,8].
- Posiphen inhibits the synthesis of APP and, therefore, inhibits accumulation of amyloid plaque through inhibition of Aβ production. Potentially it could inhibit the formation of neurofibrillary tangles by inhibiting the hyperphosphorylation of tau, and improve nerve cell survival and prevent nerve cell death through the inhibition of N-APP and C-31.
- APP can undergo aberrant processing. The holoprotein is cleaved by β secretase, with subsequent further cleavage into at least 3 toxic peptides: the N-terminal N-APP, Aβ and the C-terminal C31. All three fragments are toxic to nerve cells and are therefore called – death peptides.

Aberrant Processing Pathway: in the absence of neurotrophic factors β secretase cleaves APP at the N-terminal end of Aβ and leads to the subsequent cleavage of other toxic APP fragments.



Results

- Posiphen acts post-transcriptionally[1,9] by lowering newly synthesized APP levels, as determined by a brief 10-min incubation in the presence of ^{35}S-labeled amino acids. In contrast, both APP mRNA levels and total protein levels were unaffected (p>0.05, Dunnett) (**Figure 1**).

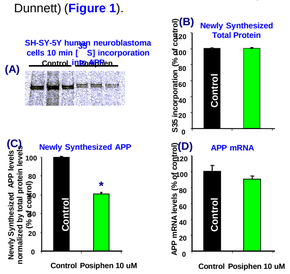


Figure 1: Translational regulation of APP by Posiphen (lowering the rate of APP synthesis) in SH–SY–5Y human neuroblastoma cells. Translation was assessed by addition of [35S]methionine and cysteine for 10 min followed by immunoprecipitation. (1) newly synthesized APP protein was reduced. (2) Newly synthesized total protein was assessed by [35S] incorporated protein levels (trichloroacetic acid precipitable counts) normalized by non–precipitable counts, and was unaffected by Posiphen (10 μM). (3) Newly synthesized APP levels were then normalized by [35S] incorporated proteins. Posiphen (10 μM) significantly decreased newly synthesized APP levels (50% reduction, p < 0.05, Dunnett). (4) APP mRNA levels were assessed by RT–PCR. Treatment with Posiphen did not affect APP mRNA levels (p > 0.05, Dunnett).

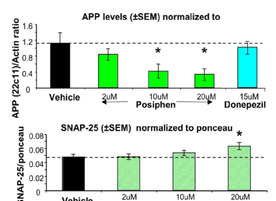


Figure 2: Posiphen actions on APP and synaptic protein in primary cortical neurons. Posiphen induced (i) a decline in extracellular APP (as assessed with mAb 22c11 normalized to β-actin levels in the same sample) and (ii) an elevation in SNAP-25 levels (mAb anti-SNAP-25 normalized to ponceau) in primary cortical neurons (*p<0.05 vs. vehicle control, Dunnett's t test). The ChEI, donepezil was without effect.

- The action of Posiphen to lower APP in cell culture translated to *in vivo* efficacy, as tested in a normal mice and a number of mice models.

- Posiphen was well tolerated and maxijmum daily doses of 150 mg/kg i.p. were tolerated (not shown). It can hence be safely administered at doses far higher than necessary to achieve brain levels that inhibit APP and Aβ.

- Posiphen is devoid of cholinergic activity. However when administered to humans and animals, Posiphen undergoes N-demethylation to generate metabolites that possess AChE activity (**Table**).

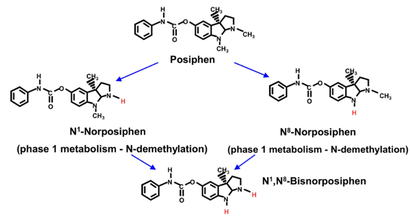
Table: Comparison of the IC_{50} values (± SEM) of anticholinesterases of clinical interest versus Posiphen and metabolites against freshly prepared human AChE and BChE.

Compound	IC$_{50}$ Value* (nM) AChE	BChE	Selectivity
Tacrine	190 ± 40	47 ± 10	4-BChE
Donepezil	22 ± 8	4150 ± 1700	188-AChE
Galanthamine	800 ± 60	7300 ± 830	9-AChE
(-)-Phenserine	22 ± 1.4	1560 ± 45	70-AChE
Posiphen	>10,000	>10,000	No activity
N^1-Norposiphen	63 ± 4.4	5300 ± 740	84-AChE
N^8-Norposiphen	5600 ± 600	1750 ± 325	4-BChE
N^1,N^8-Bisnorposiphen	230 ± 23	950 ± 110	4-AChE

*IC_{50} (concentration required to inhibit 50% enzyme activity) values were determined in duplicate on 4 occasions from freshly prepared human enzyme.

- Posiphen acts as a pro-drug that slowly generates 3 major metabolites (**Figure 3**), two - N1-Norposiphen and N1,N8-Norposiphen - are cholinergically active.

Figure 3: Structure of Posiphen and generation of primary metabolites



- The action of Posiphen to lower APP in cell culture translated to *in vivo* studies in mice following their 21 day i.p. administration (**Figure 4, Top**). Similar to cell culture studies, the reduction in APP in the brain was dose dependent and leveled off at 50% to 60% – with greater drug doses providing no additional action.

- Posiphen loweres levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brain of treated animals (**Figure 4, center and bottom**). A concentration-dependent 50% maximal decline in Aβ was achieved with a daily dose of 15 mg/kg i.p. Posiphen, with greater drug doses providing no further action. $A\beta_{1-42}$ proved more sensitive to Posiphen than $A\beta_{1-40}$. The correlation coefficients for Posiphen treatment effects on $A\beta_{1-40}$ levels vs. $A\beta_{1-42}$ levels are significantly different from each other by Fisher's Z–test (p < 0.05).

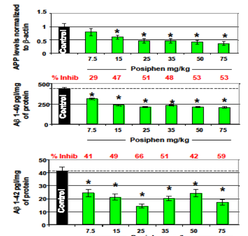


Figure 4: Analysis of brain APP and Aβ levels from posiphen treated mice. Following once daily, 21 day consecutive i.p. administration of Posiphen or saline (control) to mice, animals were killed approx 90 min after the final dose and brain samples were immediately frozen (-70ºC). Brain extracts were run on SDS–PAGE, transferred to nitrocellulose membrane, and probed with antibodies to APP (mAb 22c11) or β-actin. Data are mean ± SEM (n = 8 - 9). APP levels were normalized to β-actin levels that were unaffected by drug treatment. There was a log–linear relationship between Posiphen dose and adjusted APP levels in mouse brain. Natural log of Posiphen dose was compared to adjusted APP levels by linear regression. Regression was significant at p < 0.01 for both coefficients. The same samples were analyzed for Aβ levels (ELISAs: $A\beta_{1-40}$ and $A\beta_{1-42}$ using IBL kits Immuno–Biological Laboratories Co., Gunma, Japan), as described.[8-10] Results from all experiments were adjusted to brain tissue mass (* p < 0.05 vs. control (Dunnett).

- Posiphen readily enters the brain following systemic administration having a Clog P value similar to centrally active drugs such as valium. In a number of different mouse models it showed a brain to plasma ratio of 10:1 **Figure 5.**

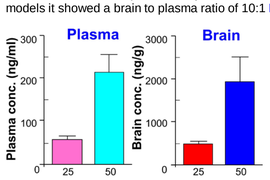


Figure 5: Plasma and brain levels of Posiphen following systemic administration. Posiphen was administered to mice by the i.p. route. Animals were killed 90 min thereafter (the time of peak concentrations), brain tissue was taken and analyzed for Posiphen.

- Posiphen improves stem cell survival, migration and differentiation into nerve cells rather than glia and astrocytes[11].

- APP23 mice can over-express by 7-fold the mutated human APP751 gene in the brain, with Aβ plaque-like deposits that begin to appear in the hippocampus and neocortex from 6 months on.

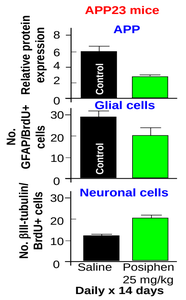
- Neural stem cells (NSCs) exposed to high concentrations of sAPP *in vitro* differentiate into mainly astrocytes, suggesting that pathological alterations in APP processing during neurode-generative conditions such as AD may prevent neuronal differentiation of NSCs.

Figure 6: Posiphen lowers APP levels in the brain of transgenic APP23 mice without lowering mRNA levels

Improves stem cell survival in brain

Increases stem cell migration to the hippocampus and cortex

Supports stem cell differentiation into neurons rather than into astrocytes



- Down syndrome (DS), the most common genetic cause of mental retardation, is due to the presence of an extra copy of human chromosome 21. The Mobley lab took advantage of the synteny between mouse chromosome 16 and human chromosome 21 and the ability to engineer mice that contain an extra copy of genes homologous to those on HSA 21; the Ts65Dn mouse12. Among the most salient findings was a striking decrease in NGF transport from hippocampus to BFCNs that was linked to degeneration of these neurons and App overexpression

Posiphen lowers App levels by 30% in brain of Ts65Dn mice, a model of trisomy Down Syndrome
Posiphen achieves a 10:1 ratio of brain to plasma levels
Brain AChE levels were significantly lowered (50% at Posiphen 50 mg/kg): verifying that Posiphen 's metabolites provide cholinergic activity

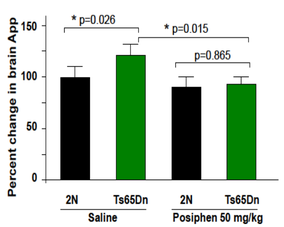


Figure 7: App levels in the hippocampus of control (2N) and Down Syndrome (Ts65Dn) mice were assessed by Western blot (App 8717 antibody). In saline treated animals, App was significantly elevated (24%) in Ts65Dn hippocampus vs. 2N controls (p=0.026). By contrast, 50 mg/kg Posiphen (i.p. x 21 days) significantly lowered App levels by 30% in Ts65Dn mice to levels that were no different from 2N controls. Separately, Posiphen lowered App levels by 16% in 2N control mice vs. saline controls *(ICAD 2008 poster, Salehi et al., Dept. Neurology)*

- Oral administration of Posiphen, similar to the i.p. route, dramatically lowered brain levels of $A\beta_{1-42}$ treated mice (**Figure 8.1**) without adverse effects. Mice were dosed with 25, 50 and 75 mg/kg/day and treated for 7 or 21 days.

- Two phase 1 ascending single and multiple dose clinical safety studies were conducted with Posiphen. In the single dose study the no effect dose was 80 mg, whereas side effects appeared at 160 mg. In the multiple dose study Posiphen was administered QID and mild side effects were seen with 240 mg/day, whereas 160 and 80 mg/day looked normal.

- Plasma samples from mice that showed lowered levels of $A\beta_{1-42}$ were compared to plasma samples from humans to extrapolate the potential efficacy of Posiphen ifrom mice to humans (**Figure 8.1**). The plasma levels that corresponded to a 50% to 60% reduction in $A\beta_{1-42}$ in mice were much lower than the Posiphen plasma concentrations achieved in a recent phase 1 clinical safety study of Posiphen in healthy men and women dosed at 80 mg/day (**Figure 8.2**) indicating that Aβ-lowering concentrations of Posiphen are achievable at no effect levels in humans.

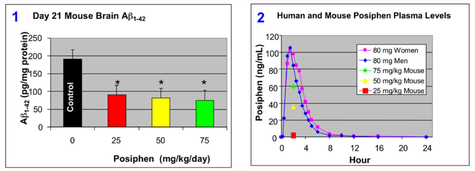


Figure 8: (1) Mouse brain levels of $A\beta_{1-42}$ are lowered by 21 day oral administration of Posiphen. Relative to vehicle control, Posiphen 25, 50 and 75 mg/kg/day oral dosing reduced mean brain $A\beta_{1-42}$ levels by 52.3%, 56.9%, and 60.2%, respectively (p < 0.01, Dunnetts). **(2)** Mean mouse Posiphen plasma concentrations at the time of brain sample collection were lower than maximum mean Posiphen plasma concentrations (determined by LC-LC-MS) in a recent clinical pharmacology study of Posiphen in healthy men and women. These healthy volunteers received a single oral dose of 80 mg Posiphen that was without adverse actions.

Conclusion

- Posiphen appears to be a promising experimental drug for AD because it can effectively lower brain levels of APP via reduction in APP synthesis. This is a post-transcriptional process which is mediated at the level of translation. Total APP mRNA and total protein synthesis remain unaffected, and our prior studies have implicated a specific region of the 5'-untranslated region (5'-UTR) of APP mRNA. This region is highly conserved across species, and APLPs do not share homology at the level of their 5'-UTR.[13]

- Posiphen inhibits APP/$A\beta_{1-42}$ in rodents following i.p. and oral administration. Maximal APP/Aβ reductions of 50% were achieved in neuronal cell cultures and in a number of mice models. Greater doses appeared to provide no additional action and plasma drug concentrations associated with this action are readily achievable in humans at well-tolerated doses.

- Through inhibition of APP Posiphen also has the potential to lower the levels of hyperphosphorylated tau, N-APP and C31.

- Posiphen, different from available ChEIs, slowly generates anticholinesterase actions via its N-demethylated metabolites in the manner of a pro-drug to potentially provide symptomatic relief.

Current & Future Focus

Our aim is to develop Posiphen into a disease modifying drug to treat AD. Inhibition of APP synthesis should potentially lead to inhibition of not just Aβ, but also of several toxic fragments cleaved from APP. We, therefore, want to test levels of N-APP and C31 before and after Posiphen treatment to see if inhibition of APP enhances the disease modifying effect of Aβ inhibitors. We believe that Posiphen may optimally combine actions on APP (to slow disease progression) with AChE (to symptomatically augment cognition) and want to move it rapidly into proof of efficacy clinical trials.

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